



TABLE I: Oligodeoxynucleotides

A. Thermodynamics and Kinetics

14-dC-S	ATG CAG CTA AGT CA
14-dC-A	TGA CTT AGC TGC AT
14-BrdC-S	ATG CAG CTA AGT CA
14-BrdC-A	TGA CTT AGC TGC AT
14-MedC-S	ATG <u>CAG</u> CTA AGT <u>CA</u>
14-MedC-A	TGA <u>CTT</u> AGC <u>TGC</u> AT
12-dC-S	CAT GCA GCC CCA
12-dC-A	TGG GGC TGC ATG
12-BrdC-S	CAT GCA GCC CCA
12-MedC-S	<u>CAT GCA</u> <u>GCC</u> <u>CCA</u>
16-dC-A	TGG GGC TGC ATG GCG T
16-BrdC-S	ACG CCA TGC AGC CCC A

Nomenclature: Oligodeoxynucleotide length - (dC, MedC, BrdC) - (S = sense; A = antisense). C = 5'-Bromodeoxycytidine, C = 5'-Methyldeoxycytidine.

B. Linkers, Displacers and Targets

PstI

GAT GAT GAT GTG <u>CAG</u> CCA ATG CCC <u>CAG</u> GAG <u>CCC</u> T	P-D-MedC
GAT GAT GAT GTG <u>CAG</u> CCA AAG CCC <u>CAG</u> GAG <u>CCC</u> T	P-D-BrdC-E(10)
GAT GAT GAT GTG CAG CCA ATG CCC <u>CAG</u> GAG <u>CCC</u> A	P-D-BrdC-E(24)
GAT GAT GAT GTG CAG CCA ATG CCC <u>CAG</u> GAG <u>CCC</u> T	P-D-BrdC
G TAC CTA CTA CTA C	P-L-dC
*G CCA ATG CCC CAG GAG CCC T	P-T-D
AC GTC GGT TAC GGG GTC CTC GGG A	P-T-L

EcoRI

CCT CGA AGG AGC CTT CCA CAG CCG AAT TGT AGT AGT AGT AAG CT	E-D-BrdC
CA TCA TCA TCA T	E-L-dC
*CCT CGA AGG AGC CTT CCA CAG CCG	E-T-D
GGA GCT TCC TCG GAA GGT GTC GGC TTA A	E-T-L

XmaI (SmaI)

TCT CGG CTC ACT GCA ACG TCC GCC TCC CGG GTA GTA GTA GTA	X-D-BrdC
CAT CAT CAT CAT	X-L-dC
CC CAT CAT CAT CAT	Sm-L-dC
*TCT CGG CTC ACT GCA ACG TCC GCC TC	X-T-D
AGA GCC GAG TGA CGT TGC AGG CGG AGG GCC	X-T-L

SfiI

GTA GTA GTA CCC CGG CCA CAC ACA CAC ACA CAC GA	S-D-BrdC
CAT CAT CAT CAT	S-L-dC

NotI

CTC TCT CTC TCT GCG GCC GTA GTA GTA	N-D-BrdC
CAT CAT CAT CAT	N-L-dC

Nomenclature: Oligodeoxynucleotides are named x-y-z where:

x= restriction site (first letter only)

y= function (displacer, linker or target)

z= composition (BrdC, MedC, dC) or polarity (displacer or linker side)

E(N) = error at position N (a mismatch) if present

C. Plasmid pMS19 construction

*Eco*RI

AAT TGA GAT CTG GCC ACC CCG GGG ACA CAC ACA CAC ACG
CT CTA GAC CGG TGG GGC CGG TGT GTG TGT TGC TTA A

PMS-ES
PMS-SE

*Hind*III

AGC TTG CTC TCT CTC TCT GCG GCC GCT TTT AGA TCT C
AC GAG AGA GAG AGA GAG CGC CGG CGA AAA TCT AGA G TCG A

PMS-NH
PMS-HN

Nomenclature: Oligodeoxynucleotides are named x-y where:
x= function (PMS)
y= orientation in product (e.g. ES is *Eco*RI to *Sfi*I)

D. Triplex-formation (at *Sall* in pMS19)

TCTCTCTCTCTCTCTC C AAGCTT GCATGC CTGCAG GTCGAC TCAATGATTCCC
G AGTTACTAAGGG BT-D-MedC(1)-1
BT-L-dC-1

C AAGCTT GCATGC CTGCAG GTCGAC TCAATGATTCCC
G AGTTACTAAGGG B0-D-MedC(1)-1
BT-L-dC-1

Nomenclature: Oligodeoxynucleotides are named x-y-z where x = BT if both branch migration and triplex formation are possible, x = B0 if only branch migration is possible; y = composition (dC, MedC or BrdC) in the branch migration region (1) and/or triplex forming region (2); z = laboratory index.



TABLE II: Bromodeoxycytidine and Methyldeoxycytidine Thermodynamics

Oligodeoxynucleotides	t_m in 1 M Na ⁺ , C = 6 μ M		
	pH 4	pH 7	pH 10
14-dC-A + 14-dC-S		57°	
14-dC-A + 14-BrdC-S		63°	
14-dC-S + 14-BrdC-A		62.5°	
14-BrdC-A + 14-BrdC-S		65°	
12-dC-A + 12-dC-S	60°	53.5°	50°
12-dC-A + 12-BrdC-S	70°	69.5°	54°
	pH 4.7	pH 7	pH 9.6
14-dC-A + 14-dC-S		57°	
14-dC-A + 14-MedC-S		60°	
14-dC-S + 14-MedC-A		59.5°	
14-MedC-A + 14-MedC-S		64.5°	
12-dC-A + 12-dC-S	50°	60°	47.5°
12-dC-A + 12-MedC-S	55°	67°	50.5°

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TABLE III: Temperature Dependence of Displacement Rates with BrdC-Containing Displacers

A. Blunt ends

Oligodeoxynucleotides	Temp (°C)	Half-time (min) for displacement with 12-BrdC-S at:		
		101 μM	20 μM	4 μM
12-dC-S* (C = 0.25 μM) +	37	2	4-8	16-32
12-dC-A (C = 0.75 μM)	32	4-8	8-16	32
	27	4-8	32-64	128-256

B. Overhangs

Oligodeoxynucleotides	Temp (°C)	Half-time (min) for displacement with 16-BrdC-S at:	
		3 μM	0.57 μM
12-dC-S* (C = 0.25 μM) +	37	<1	4-8
16-dC-A (C = 0.57 μM)	32	<1	16-32
	27	<1	2-4
* 5'- ³² P-labeled	22	<1	<1

C. Effect of Linkers

Site	Overhang	G+C%	Half time for displacement (minutes)	
			No linker	Linker
EcoRI	5'	0	60	6
PstI	3'	50	8	< 1
XbaI	5'	100	8-16	< 1

CONDITIONS: Ligase buffer (pH = 7); 37°C; 10 μL per reaction.
 Target: Kinased strand 10 ng, Unlabeled strand 30 ng.
 Displacer: 150 ng; Linker (if present): large molar excess.
 Displacer concentration = 1 μM.



TABLE IV: Linker Capture

Fragment:	34-BrdC-D		Displacer		34-MedC-D		34-dC-D	
	Experimental	Calculated	Experimental	Calculated	Experimental	Calculated	Experimental	Calculated
A	16	9		10		7		4
B	384	344		150		144		16
D	1*	1*		1*		1*		15

* by definition

TABLE V: Linker Capture with Mismatches

Displacer	Relative yield (Fragment B/Fragment A)	
	Experimental	Calculated
P-D-BrdC	24	29
P-D-BrdC-E(10)	1	1
P-D-BrdC-E(24)	24	39

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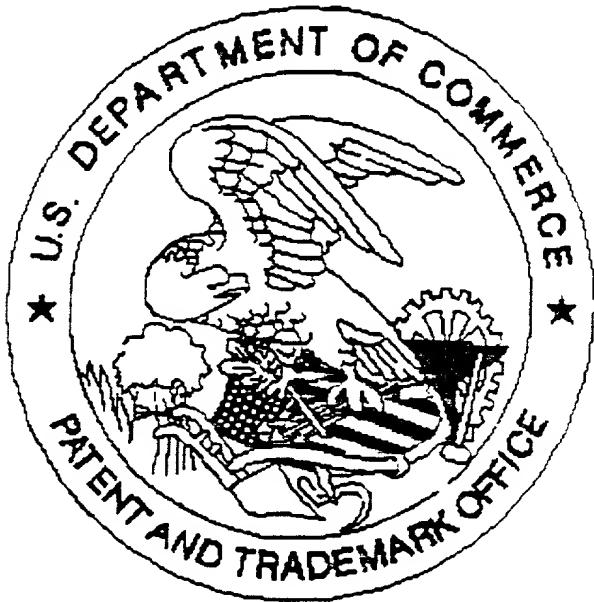
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